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TITLE: Fish Oil Supplementation and Fatty Acid Synthase
Expression in the Prostate: A Randomized Controlled
Trial

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13. ABSTRACT (Maximum 200 Words) One in seven men over age 60 will be diagnosed with prostate cancer. Elucidation of early cellular changes that may predict progression to prostate cancer and the identification of factors that may inhibit or reverse these cellular changes would be of great clinical significance. Dysregulation of lipid metabolism is an early cellular change that has recently come under investigation. Two lipid pathways will be explored in this study; 1) over-expression of the lipogenic enzyme fatty acid synthase (FAS) and 2) cholesterol accumulation in the specialized plasma membrane lipid rafts. Lipid rafts are rich in proteins that mediate signal transduction and are markers for aggressive prostate cancer. Cell culture research has demonstrated that dietary supplementation with polyunsaturated fatty acids, particularly ω -3 fatty acids, decreases expression of FAS and may alter the integrity of lipid raft formation. Treatment with cholesterol-lowering drugs, statins, has also been shown in animals to inhibit lipid raft formation and induce tumor cell death. We will conduct a randomized placebo-controlled study to evaluate the effect of fish oil, statin use and fish oil plus statin versus placebo on FAS expression and lipid raft composition in benign, pre-neoplastic and neoplastic prostate tissue from men undergoing repeat prostate biopsy.				
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INTRODUCTION

SPECIFIC AIMS:

The proposal aims have been modified and approved, as of 8 September 2004. Please see the attached DOD *Statement of Work* and *Revised Statement of Work*. We propose to conduct a double-blind, placebo-controlled, randomized intervention study using a factorial design to evaluate the effects of Fish Oil (FO) supplementation and statin use on markers of alteration in lipid metabolism in prostate tissue samples. The primary endpoints of this trial are fatty acid synthase expression, caveolin-1 expression, changes in lipid raft fractions in the plasma membrane and cell proliferation (Ki-67 expression) in benign, pre-neoplastic and neoplastic prostate tissue. The secondary endpoints include measuring the expression of SREBP-1, a transcription factor for fatty acid synthase, cell death (apoptotic fraction using TUNEL), RBC fatty acid concentration and change in PSA. Our study population will be recruited from men at the PVAMC urology clinic who are scheduled for a repeat biopsy. These men will have had an initial negative biopsy yet are still considered at high risk due to continued elevated prostatic specific antigen (PSA >4ng/ml) and/or suspicious findings by DRE or TRUS. Patient recruitment will be on-going for 33 months. Assuming a recruitment rate of 73%, we should be able to recruit a total of 144 men or approximately 36 men per arm. While this study population is limited to men at high risk of disease, the results may be more broadly generalizable to any man considered at risk of prostate cancer due to standard clinical indicators such as a PSA >4ng/ml.

BODY

During FY01 this award has supported study coordination, local and federal human subjects review, subject recruitment and data collection to address our primary aims.

HUMAN SUBJECTS REVIEW: We successfully developed and obtained supplemental DOD support to add a statin arm to our originally funded trial of FO on 2 October 2004. Local human subjects documentation was submitted for the original study on 23 September 2003 and fully approved on 12 December 2003. Two amendments to the protocol were locally approved before we submitted all approvals to the DOD HSSRD; the latter was dated 30 April 2004. Human subjects materials for the original FO study were submitted to DOD HSSRD on 21 May 2004 and we received final approval on 22 October 2004.

The protocol revisions to add a statin arm were submitted for local human subjects review on 20 August 2004 and were approved on 22 December 2004. Because this is a cancer related study, we determined we must also receive Oregon Health and Sciences University Cancer Institute's (OHSU CI) Clinical Research Review Committee (CRRC) approval. The CRRC's approval has less to do with human subjects monitoring than with any cancer-related study's scientific integrity. We submitted all fish oil and statin study-related documents to the OHSU CI CRRC on 10 November 2004 and received their final approval on 10 January 2005.

All of the above statin-related approvals and additional materials were submitted to DOD HSSRD on 23 November 2004. Additionally, we submitted pre-review responses to our reviewer's initial questions on 3 January 2005 and the ensuing amendments and local VA IRB approvals as a result of the OHSU CI's approval on 7 February 2005. We are currently awaiting DOD HSSRD review and approval.

STUDY MATERIALS: We successfully obtained a supply of the FO supplements from DSM Nutritional Products, Inc. and an even greater supply of olive oil placebos from Perfect Source. Because of the large number of olive oil capsules we have received, we will be able to use the

olive oil as placebos for the statin arm of this study as well. Upon DOD HSSRD approval for the statin arm of this study, the Portland VA Medical Center (PVAMC) Research Pharmacy has agreed to encapsulate the study drug, simvastatin, and the olive oil placebos in a different color than the FO capsules and related olive oil placebos.

STUDY COORDINATION: A study tracking system has been completed, is password protected, exists on a protected network server and is in use. Procedures have been explained to all involved entities. Dr. Mark Garzotto and Laura Peters, RN are successfully screening biopsy patients, research assistants Amy Palma and Gretchen Luhr are able to make recruitment phone calls to potential subjects, and study coordinator, Paige Farris, has been conducting the visits, recording data and completing all paperwork on each participant. Ms. Farris is also minimally tracking those who refuse so they are not approached for recruitment in the future. We have reviewed and optimized the protocol with the phlebotomists, nutritionists and laboratory technicians at OHSU's General Clinical Research Center (GCRC). All blood specimens are processed at the time of draw and are stored at -80°C . Tissue samples from subjects' initial prostate biopsy are stored in the pathology archive at the PVAMC.

PROGRESS TO DATE: Because start up has been slow, due to the extra DOD human subjects oversight process, we have successfully recruited a total of 3 subjects for the fish oil arm of this study as of February 2005. However, the study has been introduced to a total of 9 men. Two men did not meet eligibility criteria, two men refused to participate and two others are scheduled for their first visits in March 2005. Ms. Farris has conducted second visits for the first two participants and the first visit for the third participant. Each participant has completed the Diet History and Risk Factor Questionnaires, two Adverse Events questionnaires (baseline and one monthly follow-up), the Changes to Diet, Rx, Supplementals and Herbal Remedies questionnaire and has remembered to bring their unused capsules back to be counted. Completed diet history and risk factor questionnaires are scanned into a Teleform® database.

The statin arm has not yet begun. Although, the consent form and protocols have been modified to reflect the addition of the statin arm and the collection of additional biopsy cores from each participant. We have received PVAMC IRB and Oregon Health and Sciences University Cancer Institute (OHSU CI) approval for the statin arm. Additionally, urologists at OHSU have expressed an interest in conducting this study with OHSU patients (it is currently exclusively open to VA patients). As a result of the slow start up for this study as well as the strongly expressed interest, we are in the very early stages of acquiring OHSU IRB approval for this proposal. Upon DOD HSSRD approval, we will be able to start using the consent form and review the updated protocol with the GCRC staff, Dr Garzotto, Nurse Peters, interested OHSU counterparts, Ms. Farris, Ms. Palma, Ms. Luhr, the PVAMC Research Pharmacy, the OHSU Pharmacokinetics Core and the PVAMC laboratory staff.

Immunohistochemistry for fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP-1) in the blood samples will begin late in year 02, assuming adequate subject recruitment.

KEY RESEARCH ACCOMPLISHMENTS: None to date

REPORTABLE OUTCOMES: None to date

CONCLUSIONS: The primary outcome of this first year is our success in obtaining human subjects approvals for the fish oil part of the trial, obtaining supplemental funding for the

addition of a statin arm to our trial and the initiation of recruitment of veterans to participate in this study. Study procedures have been fully developed and tested for implementation. Dr. Shannon's on-going diet and prostate cancer risk study demonstrated a capacity to collect, process and store large amounts of questionnaire and biologic specimen data. We have therefore been able to build upon and benefit from this established infrastructure. We are using the same diet history and risk factor questionnaires, therefore, the Teleform® data entry programs and the DHQ nutrient analysis program have been tested. We added two other questionnaires per month for each participant, and these, too, have been tested within the Teleform® data entry programs. We separated part of the DHQ into the Changes to Diet, Rx, Supplementals and Herbal Remedies questionnaire and designed the Adverse Events Questionnaire to reflect the needs of the FO and statin trial. We will process, store and retrieve blood specimens for analysis.

Our greatest difficulty has been obtaining DOD HSSRD approvals. We have developed an ongoing dialogue with our HSSRD contact and maintain regular discussion regarding approval documents. Additionally, while waiting for the approval process to be complete, we have spent time preparing and testing our study materials. We have separated the statin and fish oil arms of the trial to allow for initial recruitment into the approved fish oil arm.

During the next year of support (3/2005 – 2/2006), we will continue subject recruitment, and begin tissue specimen processing for fatty acid synthase and phospholipid membrane lipid rafts. Based on recruitment into previous studies using patients from the PVAMC urology clinic, we estimate recruiting approximately 80% of eligible men (Garzotto, M. personal communication). An estimated 8 men undergo re-biopsy per month. Based on our pilot data, we estimate that 30% of these patients will be using statins at the time of their initial biopsy and will be ineligible for inclusion. Thus, we estimate that monthly, 6 eligible patients will be scheduled for repeat biopsy, or 198 over 33 months. Assuming a recruitment rate of 73%, we should be able to recruit a total of 144 men or approximately 36 men per arm. Power calculations are based upon a sample of 36 men per arm. Please see attached *Inclusion Enrollment Report*.

Although this is a small, randomized controlled trial investigating the effect of fish oil and statins on biomarkers of prostate cancer risk, it will provide valuable and unique data clarifying the role of the lipid metabolism pathway in chemoprevention among men at high risk for prostate cancer. Continued recruitment and randomization of subjects into this trial is imperative to our eventual success.

C. Statement of Work

Fish Oil Supplementation and Fatty Acid Synthase Expression in the Prostate: A Randomized Controlled Trial

<i>Task 1.</i>	Finalize clinical protocol and training:	Months 1-4
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- a. Develop tracking system for recording patient recruitment, contact and consent information.
- b. Obtain IRB approval from both Portland VA Medical Center and Oregon Health and Sciences University.
- c. Finalize encapsulation procedure and obtain treatment and placebo capsules.
- d. Finalize and review clinical protocol with GCRC nursing staff.
- e. Review and optimize blood processing procedures with laboratory staff.
- f. Review procedures for patient contact and recruitment with Mark Garzotto, MD, Laura Peters, RN and study coordinator, Amy Palma.

Expected Product: Tracking system, IRB approval

<i>Task 2.</i>	Subject recruitment and data collection	Months 4 – 28
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- a. Patients who have had a negative prostate biopsy in the previous two weeks and who have been scheduled by Dr. Garzotto to undergo a repeat biopsy will be recruited for our proposed trial.
- b. Initial telephone contact and schedule appointment.
- c. 1st visit – at the OHSU GCRC
 - Review the study purpose, protocol, exclusion criteria and consent documents.
 - Complete the diet history questionnaire, risk factor questionnaire.
- d. Randomization
- e. 2nd visit -- at the OHSU GCRC
 - Complete history and physical exam, 10 ml blood specimen.
 - Four week supply of placebo or treatment capsules distributed.
- f. 3rd visit – at the OHSU GCRC
 - Four week supply of placebo or treatment capsules distributed.
 - Complete side-effects and adverse events reporting form.
- g. 4th visit – at the OHSU GCRC
 - Four week supply of placebo or treatment capsules distributed.
 - Complete side-effects and adverse events reporting form.
- h. 5th visit – at PVAMC clinic area E
 - Return unused capsules.
 - Obtain 10 ml blood specimen
 - Repeat biopsy conducted per standard clinical procedure
(this is not a study linked event)

Expected Product: Questionnaire, blood specimen and biopsy data for 80 patients

Task 3. Preparation for Immunohistochemistry (IHC) / Data Entry. Months 13-24

- Optimize IHC for fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP-1)
- Develop database for tracking specimen receipt and analysis
- Begin data entry of questionnaire forms and event reporting forms

Expected Product: High functioning antibodies and procedures for FAS and SREBP-1 IHC, laboratory database, complete questionnaire data entry.

<i>Task 4.</i>	Laboratory / Dietary Analyses.	Months 24-30
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- a. Blood specimens shipped to Seattle for red blood cell fatty acid analyses.
- b. Initial and repeat biopsy specimens obtained from PVAMC pathology
- c. Perform IHC for FAS and SREBP-1 on pre and post intervention tissue specimens.
- d. Perform IHC for Ki-67 and TUNEL assay on post-intervention tissue specimens.
- e. Run nutrient analysis program on diet history questionnaire data.
- f. Data cleaning.

Expected Product: Complete data on FAS and SREBP-1 expression in 160 tissue specimens from 80 patients. Complete data on Ki-67 expression and TUNEL for 80 tissue specimens from 80 patients. Red blood cell fatty acid concentrations from 160 blood specimens from 80 patients. Nutrient intake data from 80 patients.

<i>Task 5.</i>	Final Analyses and Report Writing	Months 30-36
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- Final analysis of data from questionnaires, blood specimens and tissue specimens will be performed
- Prepare final report and initial manuscripts.

Expected Product: Completed and submitted final report a minimum of 1 submitted manuscript.

C. Revised Statement of Work

Fish Oil Supplementation and Fatty Acid Synthase Expression in the Prostate: A Randomized Controlled Trial

Task 1. Finalize clinical protocol and training: Months 1-6

- a. Develop tracking system for recording patient recruitment, contact and consent information.
- b. Obtain IRB approval from both Portland VA Medical Center and Oregon Health and Sciences University.
- c. Finalize encapsulation procedure and obtain treatment and placebo capsules.
- d. Finalize and review clinical protocol with GCRC nursing staff.
- e. Review and optimize blood processing procedures with laboratory staff.
- f. Review procedures for patient contact and recruitment with Mark Garzotto, MD, Laura Peters, RN and study coordinator, Amy Palma.
- g. Modify tracking system, protocol, and consent form to allow for the addition of a statin arm and a statin+fish oil arm.
- h. Modify tracking system, protocol, and consent form to allow for the collection of an additional prostate biopsy core to be cryopreserved.

Expected Product: Tracking system, IRB approval, IRB approval of amendment.

Task 2. Subject recruitment and data collection Months 6 – 28

- a. Patients who have had a negative prostate biopsy in the previous two weeks or patients who have had a positive biopsy but who have chosen no therapy (watchful waiting) and who have been scheduled by Dr. Garzotto to undergo a repeat biopsy will be recruited for our proposed trial.
- b. Initial telephone contact and schedule appointment.
- c. 1st visit – at the OHSU GCRC
 - Review the study purpose, protocol, exclusion criteria and consent documents.
 - Complete the diet history questionnaire, risk factor questionnaire.
- d. Randomization
- e. 2nd visit -- at the OHSU GCRC
(note: this visit may be combined with visit 1 to reduce subject burden)
 - Complete history and physical exam, 10 ml blood specimen.
 - Four week supply of placebo or treatment capsules distributed.
- f. 3rd visit – at the OHSU GCRC (for subjects residing outside the Portland area, this visit may be replaced by a telephone contact and mailed supplements)
 - Four week supply of placebo or treatment capsules distributed.
 - Complete side-effects and adverse events reporting form.
- g. 4th visit – at the OHSU GCRC (for subjects residing outside the Portland area, this visit may be replaced by a telephone contact and mailed supplements)
 - Four week supply of placebo or treatment capsules distributed.
 - Complete side-effects and adverse events reporting form.
- h. 5th visit – at PVAMC clinic area E
 - Return unused capsules.
 - Obtain 10 ml blood specimen
 - Repeat biopsy conducted per standard clinical procedure (this is not a study linked event)
 - Obtain one additional biopsy core for cryopreservation and analyses of lipid raft fractions. In men with known prostate cancer this core will be taken, if possible, from

the quadrant farthest from the known tumor. Fresh tissue collected at surgery by study RA and delivered immediately to the OHSU Pharmacokinetics Core for cryopreservation.

Expected Product: Questionnaire, blood specimen and biopsy (frozen and paraffin embedded) data for 120 patients.

Task 3. Preparation for Immunohistochemistry (IHC) / Data Entry. Months 13-24

- a. Optimize IHC for fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP-1)
- b. Optimize protocol for lipid raft extraction from tissue specimens (using stored non-study tissue).
- c. Develop database for tracking specimen receipt and analysis
- d. Begin data entry of questionnaire forms and event reporting forms

Expected Product: High functioning antibodies and procedures for FAS and SREBP-1 IHC, final procedures for lipid extraction and raft associated protein analyses, laboratory database, complete questionnaire data entry.

Task 4. Laboratory / Dietary Analyses.

Months 24-32

- a. Blood specimens shipped to Seattle for red blood cell fatty acid analyses.
- b. Initial and repeat biopsy specimens obtained from PVAMC pathology.
- c. Perform IHC for FAS and SREBP-1 on pre and post intervention tissue specimens.
- d. Perform IHC for Ki-67 and TUNEL assay on post-intervention tissue specimens.
- e. Perform lipid extraction and mass spectrometry for identification of raft associated proteins and lipids.
- f. Run nutrient analysis program on diet history questionnaire data.
- g. Data cleaning.

Expected Product: Complete data on FAS and SREBP-1 expression in 240 tissue specimens from 120 patients. Complete data on Ki-67 expression and TUNEL for 120 tissue specimens from 120 patients. Complete data on quantity of raft associated long chain fatty acids and caveolin-1 from 120 frozen biopsy specimens. Red blood cell fatty acid concentrations from 240 blood specimens from 120 patients. Nutrient intake data from 80 patients.

Task 5. Final Analyses and Report Writing

Months 32-36

- a. Final analysis of data from questionnaires, blood specimens and tissue specimens will be performed
- b. Prepare final report and initial manuscripts.

Expected Product: Completed and submitted final report a minimum of 1 submitted manuscript.

**Inclusion Enrollment Table: Projected Accrual for the Present Study
(actual estimates, not percentages).**

Ethnic Category	Sex/Gender			
	Females	Males	Unknown	Total
Hispanic or Latino		12		12
Not Hispanic or Latino		132		132
Unknown				
Ethnic Category: Total of all subjects*		144		144
Racial Category				
American Indian or Alaskan Native		2		2
Asian		4		4
Black or African American		2		2
Native Hawaiian or other Pacific Islander		1		1
White		125		125
More than one race				
Unknown				
Racial Category: Total of all subjects*		144		144

*These totals must agree.